MINIREVIEW

MicroRNAs and HIV-1: Complex Interactions*

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RNAi plays important roles in many biological processes, including cellular defense against viral infection. Components of the RNAi machinery are widely conserved in plants and animals. In mammals, microRNAs (miRNAs) represent an abundant class of cell encoded small noncoding RNAs that participate in RNAi-mediated gene silencing. Here, findings that HIV-1 replication in cells can be regulated by miRNAs and that HIV-1 infection of cells can alter cellular miRNA expression are reviewed. Lessons learned from and questions outstanding about the complex interactions between HIV-1 and cellular miRNAs are discussed.

RNAi is a biological mechanism widely conserved from yeast through humans (1). A key component of the RNAi machinery is the RNA-induced silencing complex (RISC),[2] which, in its minimal form, is composed of an Argonaute protein and a small noncoding RNA (ncRNA) of ~22–30 nucleotides in length. The biogenesis of RISC and its activities have been extensively reviewed elsewhere (2, 3); simply stated, using its small ncRNA component as a guide sequence, an RNA-loaded RISC targets a complementary mRNA and elicits gene silencing via RNA degradation or a reduction in mRNA translation (4–6).

There are three major types of small ncRNAs: Piwi-interacting RNA (piRNA), siRNA, and microRNA (miRNA) (6). Mammalian piRNAs number between an estimated 50,000 and 200,000 distinct entities (7); the human genome encodes ~2000 miRNAs (miRBase). Currently, the number of endogenous siRNAs in mammalian cells remains incompletely understood and is still poorly characterized (8). RNases that participate in the generation of these small ncRNAs include: Drosha (miRNA), Dicer (siRNA and miRNA), and Argonaute (piRNA) (Fig. 1). miRNA and siRNA biogenesis has been well described (2, 3), whereas piRNA biogenesis is less well understood and in part involves the processing of single-stranded RNAs by yet identified ribonuclease(s) (9). Once assembled, piRISCs are thought to act primarily to silence cellular mobile genetic elements (*e.g.* retrotransposons and transposons) (10–12), whereas endo-siRISCs and miRISCs regulate targeted mRNAs through perfect or imperfect complementarities with sites located in the 5'-UTR (13, 14), coding (15, 16), and 3'-UTR (17, 18) sequences in the substrate RNAs.

RNAi activity is involved in many eukaryotic cellular processes. For example, the dysregulated expression of miRNAs and other RNAi components has been described in cancers, metabolic disorders, and infectious diseases (19, 20). In plants, RNAi serves as a host defense mechanism against viral infections (21, 22). In mammals, the role of RNAi in regulating viral infection is less clear; however, accumulating findings are consistent with this role (23–25), including recent evidence that the virulence of viral infection in mammals is increased when host RNAi functions are attenuated (26–30). Substantive advances have been made in our understanding of the interplay between HIV-1 and the cell's RNAi activity. Recent findings illustrate that many human miRNAs can target HIV-1 sequences and that HIV-1 infection can change the miRNA expression profiles in the circulating blood cells from infected individuals (25). Here, we briefly review extant progress and discuss future questions pertinent to HIV-1 and RNAi biology.

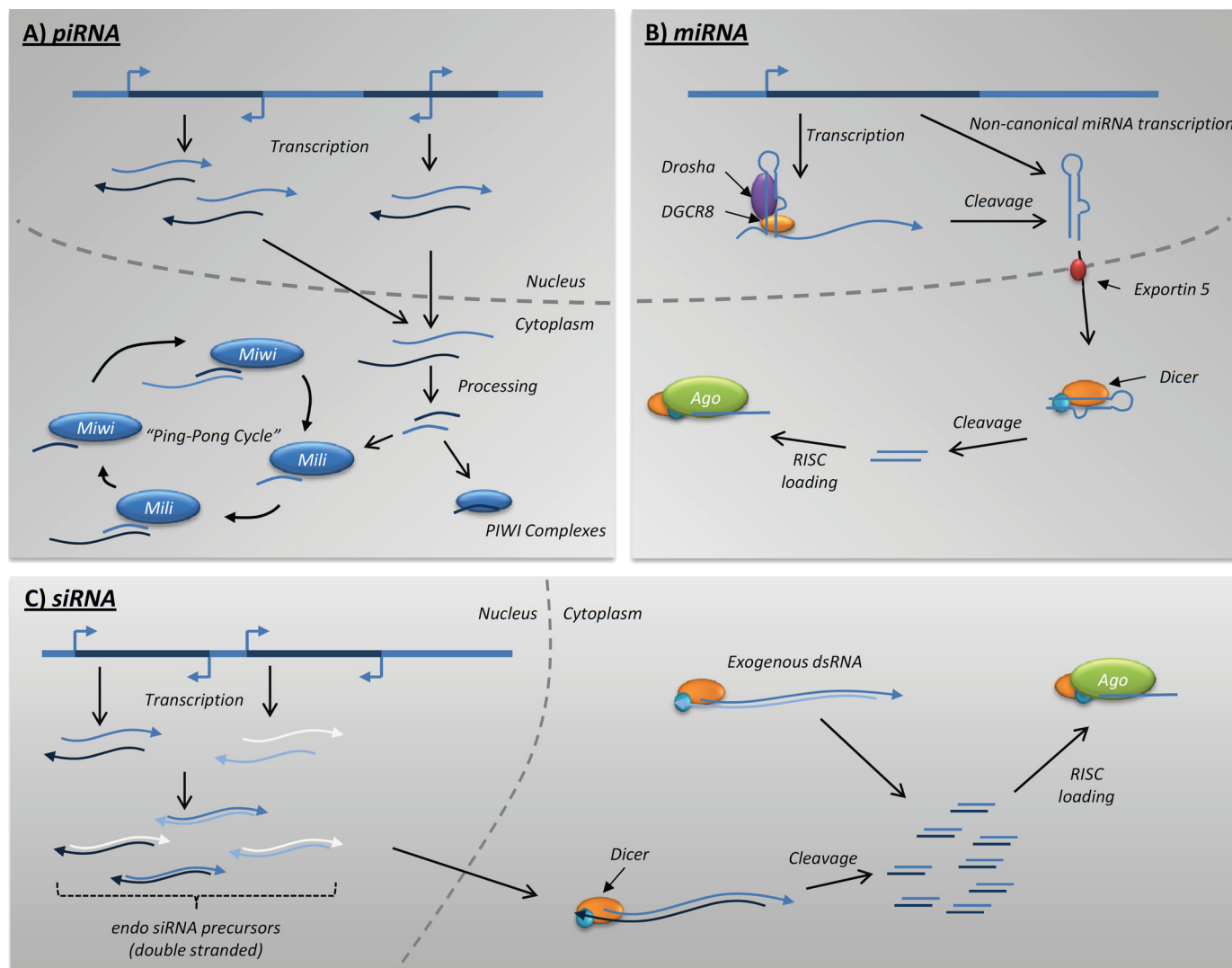## Cellular miRNAs Regulate HIV-1 Expression

miRNAs can modulate HIV replication in two ways: directly targeting HIV RNA or targeting the mRNAs that encode host cell factors relevant to HIV replication (Fig. 2 and Table 1). The first attempt to search for miRNAs that directly target sites in the HIV genome was made by Hariharan *et al.* (31). Using target prediction software, they identified five miRNA (miR-29a, miR-29b, miR-149, miR-324-5p, and miR-378) target sites in the HIV-1 genome, with two of these sites located in the viral *nef* gene (31). These investigators confirmed later that one of the five identified miRNAs (miR-29a) inhibited *nef* expression, leading to repressed HIV replication in Jurkat cells (32). This effect was also observed by Rana and colleagues (33), who predicted target sites for 11 miRNAs in the HIV-1 3'-UTR and further experimentally validated an inhibitory effect of miR-29a on HIV replication. More recently, Sun *et al.* (34) also reported the inhibition of HIV-1 infection by miR-29a and miR-29b; they noted, however, that miR-29 access to HIV-1 is limited by the complex RNA secondary structure surrounding the target site.

A different group of five miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) that target the 3'-UTR of the HIV genome was reported separately by Huang *et al.* (35). These "anti-HIV" miRNAs were shown to be enriched in resting CD4$^+$ T cells and were hypothesized to be involved in proviral latency. In a another study, Wang *et al.* (36) reported that four of the miRNAs characterized by Huang *et al.* were also responsible for differences between monocytes and macrophages in their permissivity to HIV infection. Recent work by Houzet *et al.* (37) confirmed the action of miR-29 and identified four new miRNAs that target HIV-1 (miR-133b, miR-138, miR-149, and miR-326). Taken together, the findings collectively suggest that, in divergent cells and in varying contexts, different miR-

FIGURE 1. **Biogenesis of small ncRNA.** The three main types of small ncRNA found in mammals are derived through the action of various RNases on different RNA precursors. *A*, piRNA is formed when RNA transcripts are cleaved by a still unidentified RNase before associating in the cytoplasm with a PIWI complex. This processed strand, in complex with a PIWI protein (Mili and Miwi in mammals), may directly mediate RNAi. Alternatively, the piRNA may aid in the production of additional piRNAs through what has been termed the "ping-pong cycle." piRNA guides the PIWI complex to a complementary RNA and cleaves it. The small cleaved RNA then associates with another PIWI protein and cleaves RNA complementary to it. This cycle can continue as long as pairs of complementary transcripts are available. *B*, miRNA is synthesized when long RNA transcripts fold into hairpin structures that can be recognized by the complex of the RNase Drosha and DGCR8. Drosha cleavage results in a short hairpin structure that is exported from the nucleus. These hairpins are bound by Dicer in a complex with TAR RNA-binding protein and then cleaved by Dicer in the cytoplasm to yield the mature miRNA duplex. One strand of the duplex is loaded into the Argonaute-containing RISC. *C*, a siRNA is produced when long dsRNA (from either the cell or external sources) is acted upon directly by the Dicer protein. This cleavage generates a phased array of siRNA duplexes that are loaded into the RISC. Mature siRNA can be classified as endo- or exo-siRNA based upon the origin of the original dsRNA.

NAs may selectively regulate HIV-1 infection through direct targeting of viral sequences.

miRNAs regulate host gene expression through complementarity-driven silencing of cellular mRNA sequences. Genome-wide screenings have identified recently several hundred host proteins that regulate optimal HIV-1 replication in human cells (38–42). In considering host cell proteins, it is reasonable that miRNAs may regulate them in ways that impact viral replication. There are several examples that support this reasoning. For instance, Sung and Rice (43) found that miR-198 targets the cyclin T1 mRNA, which encodes a HIV-1 Tat cofactor, to confer differences in permissiveness between monocytes and macrophages to HIV infection. They also found that, in different cellular contexts, other miRNAs regulate cyclin T1

(*e.g.* miR-27b, miR-29b, miR-223, and miR-150), potentially explaining expression differences of HIV in resting *versus* activated T cells (44). Independently, miR-17-5p and miR-20a were found by a separate group to target p300/CBP-associated factor (PCAF), another cellular cofactor of the viral Tat protein (29), and a further set of six miRNAs (miR-15a, miR-15b, miR-16, miR-20a, miR-93, and miR-106b) were reported to repress the expression of a third Tat cofactor, Pur-α, which was noted also to be enriched in monocytes (45). In a converse example, Zhang *et al.* (46) found that miR-217 was induced by Tat and that miR-217 increased HIV-1 expression by targeting SIRT-1 (sirtuin-1), a host protein that deacetylates and inactivates Tat function. Collectively, these discrete findings illustrate the complex miRNA-
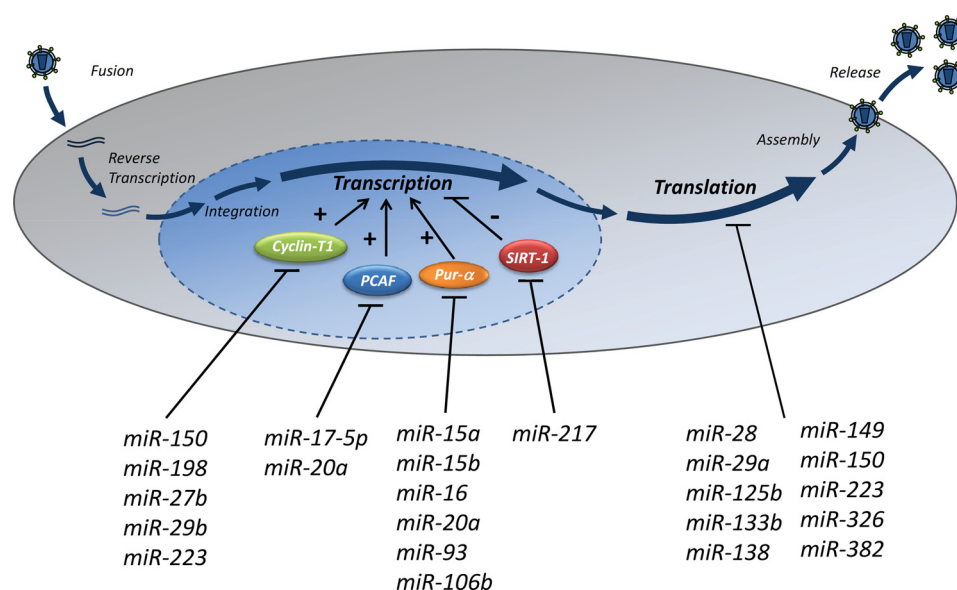
FIGURE 2. **Host miRNAs regulate HIV replication.** Host miRNAs that have been experimentally shown to regulate HIV expression are illustrated. HIV-1 transcription is altered by miRNAs that target positive (+) or negative (−) transcription factors (*e.g.* cyclin T1, PCAF, Pur-α, and SIRT-1). Other miRNAs, as outlined in text, can directly target HIV RNA and decrease viral protein expression through inhibition of translation.

TABLE 1
miRNAs involved in the regulation of HIV-1 expression

Targeting	miRNAs	Target	Function	References
DIRECT	**miR-29a**	nef	Decrease HIV infectivity	[32,33,34]
	miR-28 **miR-125b*** **miR-150** **miR-223** **miR-382**	3' LTR	Latency in primary resting CD4+ T cells	[35]
			Restrict HIV-1 replication in monocytes	[36]
	miR-133b **miR-138** **miR-149** **miR-326**	3'LTR**	Decrease HIV replication	[37]
INDIRECT	**miR-17-5p** **miR-20a**	PCAF	Decrease HIV infection	[29]
	miR-198	Cyclin T1	Restricts HIV-1 replication in monocytes	[43]
	miR-27b	Cyclin T1	Restrict HIV-1 replication in resting CD4$^+$ T cells	[44]
	miR-29b **miR-150** **miR-223**	Cyclin T1***		
	miR-15a **miR-15b** **miR-16** **miR-20a** **miR-93** **miR-106b**	Pur-α	Restrict HIV-1 replication in monocytes	[45]
	miR-217	SIRT-1	Increases HIV-1 expression in MAGI cells	[46]

* miR-125b was involved in HIV-1 restriction in resting T cells only.
** This study specifically examined only the miR-326 target site.
*** Indirect regulation of cyclin T1 by miR-29b, miR-150, and miR-223.

mediated positive and negative regulatory events that influence viral replication (Fig. 2).

HIV-1 is only one of many viruses whose expression has been demonstrated to be modulated by cellular miRNAs. Inhibition of influenza virus replication has been described for four miRNAs: miR-323, miR-491, miR-654 (47), and let-7c (48). Other examples include the targeting of primate foamy virus 1 by miR-32 (49); vesicular stomatitis virus by miR-24 and miR-93 (27); hepatitis B virus by miR-125a-5p (50), miR-199a-3p, and miR-210 (51); and hepatitis C virus (HCV) by miR-196, miR-296, miR-351, miR-431, and miR-448 (52). For HCV, a rather unique and unexpected finding is that a liver-specific miRNA, miR-122, was found (unlike other miRNAs, which generally repress viral replication) to directly target HCV RNA sequence to up-regulate viral replication (53). So far, there are no other examples of viral replication being up-regulated by direct miRNA-mediated targeting. Indeed, the list of viruses that are regulated by cellular miRNAs is expected to grow longer, and we expect to learn more about the apparently conserved role played by miRNAs in regulating the infection of cells by many viruses (54).

HIV-1 Infection Changes Cellular miRNA Profiles

As noted above, cellular miRNAs can affect viral replication; and conversely, it could be that infection by mammalian viruses alters the cell's miRNA expression (55, 56). For HIV-1, the first report of this effect was shown by the transfection of a replication-competent HIV-1 molecular genome into HeLa cells, which demonstrated repression of the majority of the cell's miRNAs (57). Initially, it was thought that the RNAi-suppressing function of the HIV-1 Tat protein (58, 59) explained these miRNA changes. However, later findings suggested more complex explanations (60). Thus, in HIV-1 infection of CEM T cells, Hayes *et al.* (60) observed changes in 145 cellular miRNAs, with the repression of only 22 cellular miRNAs appearing to correlate with the RNAi-suppressing activity of Tat. In a separate *ex vivo* HIV-1 infection of primary peripheral blood mononuclear cells (PBMCs), Sun *et al.* (34) reported reduced expression of miR-21, miR-155, miR-29a, miR-29b, miR-29c, and miR-142-3p and the increased expression of miR-223. Their observed reduction in miR-29a, miR-29b, and miR-29c agrees with similar findings obtained by HIV-1 infection of CEM T cells reported by Hayes *et al.* (60) and *ex vivo* PBMC infections reported by Houzet *et al.* (61). However, their miR-21, miR-155, miR-142-3p, and miR-223 changes were not observed in the latter two studies.

Besides miRNA changes in *ex vivo* infected cells, there is also intense interest in understanding *in vivo* miRNA changes in HIV-1-infected individuals (61). This is technically more challenging because HIV-1 infects a very small fraction of CD4^{+} T cells *in vivo*, estimated to be 1 in 10,000 in the blood and 1 in 100 in the lymph nodes (62); thus, the profile of miRNA expression in blood cells would reflect contributions from uninfected and virus-infected cells. The first investigation of this issue was made by Houzet *et al.* (61), who studied PBMCs from 36 HIV-1-seropositive individuals and compared them with corresponding samples from 12 uninfected controls. They found 59 miRNAs that were down-regulated in PBMCs from the seropositive individuals, and they noted that the pattern of miRNA changes was different depending on whether the infected individuals had high CD4^{+} T cells and low viral load, high CD4^{+} T cells and high viral load, low CD4^{+} T cells and low viral load, or low CD4^{+} T cells and high viral load. These initial findings have been followed up by two other studies. Hence, Bignami *et al.* (63) examined changes in 377 miRNAs in CD4^{+} T cells from HIV-1-seropositive patients matched with controls. They profiled miRNAs in elite HIV-1 controllers (individuals who had a documented history of infection of ~15 years and maintained a mean CD4^{+} T cell count of ~900 cells/ml with undetectable viremia in the absence of therapy), multiply HIV-1-exposed but uninfected individuals, and treatment-naïve HIV-1-infected patients. They found that the miRNA profiles from the elite HIV-1 controllers and treatment-naïve HIV-1-infected individuals were virtually indistinguishable but that they differed from the miRNA profiles in multiply HIV-1-exposed uninfected persons. The results suggested that miRNA profiling could distinguish between HIV-1-infected and HIV-1-exposed but uninfected individuals. Bignami *et al.* interpreted that the miRNA changes in HIV-1-infected persons were likely consequences of immune responses to ongoing *in vivo* replication of HIV-1, whereas the miRNA changes in the repeatedly exposed but uninfected individuals (as defined by a history of frequent sexual encounters with an HIV-infected partner) were due to immune responses from recurrent exposures to non-replicating HIV-1 antigens.

A separate *in vivo* study was performed by Witwer *et al.* (64), who compared miRNA profiles in PBMCs from healthy individuals, elite HIV-1 controllers (<50 viral copies/ml), and viremic HIV-1 patients. They observed the down-regulation of miR-150 and miR-29 family members in viremic patients, similar to the published results of Houzet *et al.* (61), and also the down-regulation of miR-150 and miR-125b, in agreement with the earlier findings of Huang *et al.* (35). Overall, Witwer *et al.* concluded that *in vivo* miRNA changes correlate, albeit imperfectly, with clinical disease parameters of CD4^{+} cell counts and plasma viral loads and that *in vivo* miRNA profiles could be useful biomarkers for HIV-1 disease progression.

The understanding of genome-wide protein and miRNA changes in HIV-1 infection is at a relatively early stage. When viewed *en toto*, the published data from the various studies could be considered to show very modest overlaps in the miRNA profiles. This degree of discordance in miRNA results mirrors similarly limited agreements among the several hundred protein factors that have been identified in multiple genome-wide surveys of host proteins that contribute to HIV-1 replication (reviewed in Ref. 41). A clear future challenge for these large data-intensive experiments is to better control the variabilities in methodology and the cell and patient conditions between the studies. Whether (and how) miRNA changes can causally affect *in vivo* HIV-1 progression or if the miRNA changes are simply consequences of HIV-1 infection also needs careful dissection.

HIV-1 Proteins That Interact with the RNAi Machinery

Although HIV-1 infection can change the expression of cellular miRNAs, it remains unclear if HIV-1 proteins act directly to influence cellular RNAi activity. Current data show that plant viruses, invertebrate viruses (65–67), and mammalian viruses, such as influenza virus, Ebola virus, and human T cell lymphotropic virus (59, 68–70), encode modulators of cellular RNAi. For HIV-1, the viral Tat protein, a transcriptional activator with a highly basic RNA-binding domain that can inhibit cellular interferon response (71, 72), has also been suggested to have RNAi-suppressing activity (58). How Tat suppresses RNAi remains incompletely understood; however, the highly basic amino acid domain of Tat (58, 73) can bind and sequester small RNAs, preventing their association with and activation of Dicer (74). The ability of Tat to suppress cellular RNAi function has been shown in several assays (59, 75, 76), including the direct delivery of Tat into neurons, which changed (by >2-fold) the expression of 50 cellular miRNAs (77). Nonetheless, it should be noted that the observed apparent RNAi activity of Tat has also been suggested to reflect an indirect effect of its transactivation of cellular promoters (78).

Two other HIV-1 proteins have also been proposed to influence cellular RNAi function. Mukerjee *et al.* (79) studied the HIV-1 Vpr protein. They showed that Vpr can cross the plasma membrane; and in tissue culture experiments, it, like Tat, can affect the properties of neuronal cells. Thus, the treatment of a neuronal cell line with soluble Vpr protein up-regulated 30 and down-regulated 15 miRNAs (79), suggesting a Vpr effect on the cellular RNAi machinery. However, a separate study that compared CEM T cell line infection with either Vpr[+] or Vpr[−] HIV-1 found no cellular miRNA changes attributable to the Vpr protein (60), questioning the influence of Vpr on RNAi. Separately and unexpectedly, Bouttier *et al.* (80) reported that HIV Gag binds the AGO2 (<u>A</u>r<u>go</u>naute-<u>2</u>) protein. Intriguingly, their results suggested that a Gag-AGO2 protein interaction does not affect RNAi function directly but enhances viral replication by increasing the packaging of HIV-1 RNAs into virions. However, it remains unclear whether the redirecting by Gag of AGO2 away from its RISC function into a novel RNA-packaging pathway might indirectly change cellular RNAi.

Future Questions and Perspectives

Our understanding of HIV-1 interaction with the host's RNAi machinery is still at a nascent stage. As we move forward, several additional questions warrant consideration. First, it remains debated whether HIV-1 RNAs are processed efficiently by host cells into viral ncRNAs. Recently, bovine leukemia virus, an animal retrovirus, was reported to synthesize a viral RNA that is proficiently processed in cells into small ncRNAs

(81). In three pyrosequencing studies of RNAs from HIV-1-infected cells (82–84), several small HIV-1 ncRNAs have been identified, some measuring at levels slightly higher compared with low abundance cellular miRNAs, suggesting that they may serve biological roles. Nonetheless, the relative paucity of abundantly expressed HIV-1 ncRNAs remains puzzling. Is this because of inefficient biogenesis or overly efficient degradation? Are HIV-1 ncRNAs preferentially degraded by yet to be characterized small RNA-degrading nucleases (85, 86)? Although answers pertaining to degradation remain unknown, there is increasing evidence that HIV-1 can express an antisense transcript from the 3′-end of its genome (87–89) that can form long RNA duplexes with counterpart sense HIV-1 RNAs. Whether these putative long antisense-sense double-stranded RNAs can be precursors for viral ncRNA biogenesis requires further examination.

Another topic of emerging interest is the role of extracellular circulating plasma miRNAs in normal and diseased states (90, 91). Compared with control plasmas, it has been reported that patient plasmas have elevated levels of miR-155 in female breast cancer individuals (92); miR-155 and miR-21 in lung cancer patients (93); and miR-21, miR-141 and miR-200 in persons with ovarian cancer (94). Relevant to disease-associated extracellular miRNAs, two recent studies have suggested that HIV-1 infection increases the release of exosomes that contain cell-free miRNAs from macrophages and peripheral blood lymphocytes (95, 96). Important future questions include how cell-free circulating miRNAs are changed in the plasma of HIV-1-infected individuals compared with uninfected controls and if these circulating miRNAs causally influence AIDS pathogenesis.

Here, we have focused our discussion on RNAi-mediated post-transcriptional gene silencing, which is a well accepted biological process in mammals. By contrast, RNAi-mediated transcriptional gene silencing (TGS) is a well characterized finding in lower eukaryotes that has remained controversial for mammals because many of the components needed for this process are found in plants and yeasts but are absent in mammalian genomes (97, 98). Currently, we do not know the role, if any, of TGS in the physiological regulation of and/or in the therapeutic utility for HIV-1. There are reports suggesting HIV-1 promoter-specific TGS (99, 100) in human cells, which will need to be carefully validated. The thoughtful investigation and integration of the roles contributed by post-transcriptional gene silencing and TGS to HIV-1 infection of cells and humans promise to keep researchers busily occupied for many years.

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